

Claims

1. A method for detecting the presence of a IgG4 polypeptide having a selected disulfide linkage pattern in a sample comprising,
 - 5 loading a sample containing a polypeptide having a selected disulfide linkage pattern, wherein the sample comprises an inhibitor of disulfide bond rearrangement, onto a chip comprising a channel having a separation medium effective to act as an obstacle to the migration of the polypeptide having a selected disulfide linkage pattern, and at least two electrodes disposed within the channel to induce an electric field,
 - 10 applying an electric field across the separation medium of the chip whereby a separation of the IgG4 polypeptide having a selected disulfide linkage pattern as compared to a IgG4 polypeptide not having the selected disulfide linkage pattern is achieved, and
 - 15 determining the presence of the IgG4 polypeptide having a selected disulfide linkage pattern.
2. A method for detecting the presence of a polypeptide having a selected disulfide linkage pattern in a sample consisting of a mixture of polypeptide multimers having two or more polypeptide chains and comprising at least one disulfide linkage between the polypeptide chains comprising,
 - 20 loading a sample containing the mixture of polypeptide multimers, wherein the sample comprises an inhibitor of disulfide bond rearrangement, onto a chip comprising a channel having a separation medium effective to act as an obstacle to the migration of the polypeptide having a selected disulfide linkage pattern, and at least two electrodes disposed within the channel to induce an electric field,
 - 25 applying an electric field across the separation medium of the chip whereby a separation of the polypeptide having a selected disulfide linkage pattern as compared to a polypeptide not having the selected disulfide linkage pattern is achieved, and
 - 30 determining the presence of the polypeptide having a selected disulfide linkage pattern.
3. The method of claim 1 or 2, wherein the inhibitor is a sulphydryl alkylating reagent.
4. The method of claim 3, wherein the sulphydryl alkylating reagent is selected from the group consisting of iodoacetamide and N-ethylmaleimide (NEM).
- 35 5. The method of claim 4, wherein the sulphydryl alkylating reagent is N-ethylmaleimide (NEM).

6. The method of claim 5, wherein the amount of N-ethylmaleimide (NEM) is between about 1mM to about 10 mM.
- 5 7. The method of claim 1 or 2, wherein the method further comprises determining the presence of a polypeptide impurity.
8. The method of claim 1, wherein the IgG4 polypeptide having a selected disulfide linkage pattern is a half-antibody.
- 10 9. The method of claim 2, wherein the polypeptide having a selected disulfide linkage is a half-antibody.
10. The method of claim 9, wherein the half-antibody is of the IgG4 class.
- 15 11. The method of claim 1, wherein the IgG4 polypeptide having a selected disulfide linkage pattern is recombinantly produced.
12. The method of claim 1 or 2, wherein the polypeptide is recombinantly produced.
- 20 13. The method of claim 1 or 2, wherein the polypeptide having a selected disulfide linkage pattern is recombinantly produced.
14. The method of claim 1, wherein, the IgG4 polypeptide not having the selected 25 disulfide linkage pattern is an anti-integrin antibody.
15. The method of claim 2, wherein the mixture comprises an anti-integrin antibody.
16. The method of claim 14 or 15, wherein the anti-integrin antibody is 30 recombinantly produced.
17. The method of claim 1 or 2, wherein the sample is obtained from the growth medium of a cell culture.
- 35 18. The method of claim 1 or 2, wherein the sample comprises about 1 to about 5000 ug/ml of a polypeptide having a selected disulfide linkage pattern.
19. The method of claim 1 or 2, wherein the separation medium is a gel polymer.

20. The method of claim 1 or 2, wherein the separation medium is non-reducing.
21. The method of claim 1 or 2, wherein the migration of the polypeptide is detected
5 using a fluorescence detector.
22. The method of claim 1 or 2, wherein the electric field is non-alternating.
23. The method of claim 1 or 2, wherein the separation further comprises isoelectric
10 focusing.
24. The method of claim 1 or 2, wherein the separation is according to the molecular weight of the polypeptide.
- 15 25. The method of claim 1 or 2, wherein the chip comprises a precast gel polymer.
26. A kit for detecting the presence of a polypeptide having a selected disulfide linkage pattern comprising, a chip and instructions for carrying out the method of claim
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27. A kit for determining the purity of a therapeutic polypeptide having a selected disulfide linkage pattern comprising, a chip and instructions for carrying out the method of claim 1.
- 25 28. The kit of claim 26 or 27, wherein the kit further comprises a component selected from the group consisting of, separation medium, non-reducing buffer, protein dye, formulation buffer, and means for inducing an electric field through a separation medium.
- 30 29. The kit of claim 26 or 27, wherein the kit further comprises instructions for determining the presence of a polypeptide impurity.
30. The kit of claim 26 or 27, wherein the kit further comprises one or more polypeptide standards.
- 35 31. A method of inhibiting disulfide bond rearrangement, wherein the polypeptide is incubated with a sulfhydryl alkylating agent selected from the group consisting of iodoacetamide and N-ethylmaleimide (NEM).

32. The method of claim 31, wherein the sulphydryl alkylating reagent is N-ethylmaleimide (NEM).

5 33. The method of claim 32, wherein the concentration of N-ethylmaleimide (NEM) is between about 1 mM to about 10 mM.

34. The method of claim 31, wherein the disulfide bond rearrangement occurs upon exposure to heat.

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35. A composition comprising a polypeptide and inhibitor of disulfide bond rearrangement, wherein the inhibitor is a sulphydryl alkylating agent.

15 36. The composition of claim 35, wherein the sulphydryl alkylating agent is selected from the group consisting of iodoacetamide and N-ethylmaleimide (NEM).

37. The composition of claim 36, wherein the sulphydryl alkylating reagent is N-ethylmaleimide (NEM).

20 38. The composition of claim 37, wherein the concentration of N-ethylmaleimide (NEM) is between about 1 to about 10 mM.

39. The composition of claim 35, wherein the polypeptide is a multimeric polypeptide.

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40. The composition of claim 39, wherein multimeric polypeptide is an antibody or half-antibody.

41. The composition of claim 40, wherein the antibody is an IgG4 antibody.

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42. The composition of claim 41, wherein the antibody is an anti-integrin antibody.

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